Valorization of Pyrolysis Liquids

Figueirêdo, Monique B.; Deuss, Peter J.; Venderbosch, Robbie H.; Heeres, Hero J.

Published in:
ACS Sustainable Chemistry and Engineering

DOI:
10.1021/acssuschemeng.8b04856

ACS Sustainable Chemistry & Engineering

Research Article

Cite This: ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

pubs.acs.org/journal/ascecg

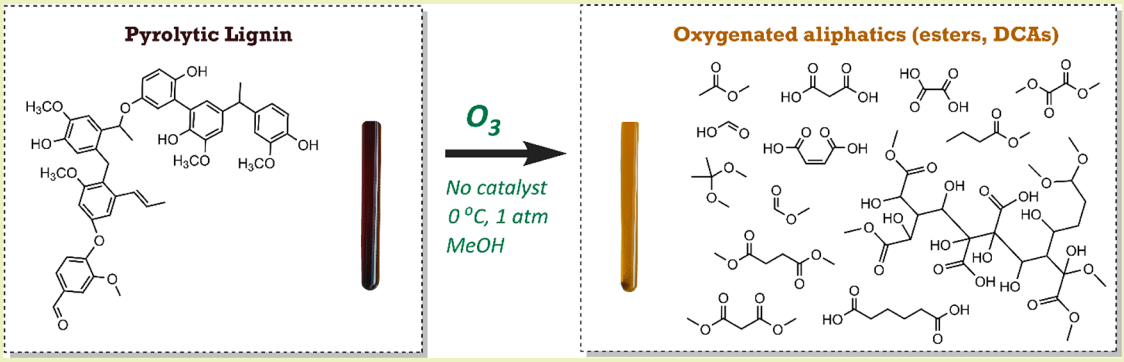
Valorization of Pyrolysis Liquids: Ozonation of the Pyrolytic Lignin Fraction and Model Components

Monique B. Figueirêdo,[†] Peter J. Deuss,[†] Robbie H. Venderbosch,[‡] and Hero J. Heeres*[,†]

[†]Department of Chemical Engineering, ENTEG, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

[‡]Biomass Technology Group BV, Josink Esweg 34, 7545 PN Enschede, The Netherlands

Supporting Information



ABSTRACT: Pyrolytic lignin is the collective name of the lignin-derived fraction of pyrolysis liquids. Conversion of this fraction to biobased chemicals is considered an attractive valorization route. Here we report experimental studies on the ozonation of a pine-derived pyrolytic lignin dissolved in methanol (33 wt %). Results show a high reactivity of ozone, and a molecular weight reduction of up to 40% was obtained under mild conditions (0 °C, atmospheric pressure) without the need for catalysts. Detailed analysis of the product mixtures (GC/MS-FID, HPLC, GPC, NMR) showed the presence of low molecular weight (di)acids and esters, along with larger highly oxygenated aliphatics. A reaction network is proposed including the heterolytic cleavage of aromatic rings, followed by secondary reactions. The observations were supported by experimental studies using representative pyrolytic lignin model compounds and a biosynthetic lignin oligomer, which aided further elucidation on the reactivity trends for different chemical functionalities. Accordingly, the presence of hydroxy and methoxy substituents on the aromatic rings is shown to be the main reason for the high reactivity of pyrolytic lignin upon ozone exposure.

KEYWORDS: *Ozonation, Oxidation, Pyrolytic lignin, Biobased chemicals*

INTRODUCTION

Environmental concerns related to the use of fossil resources have stimulated research on the use of biomass as a renewable resource. Within this context, the development of efficient, techno-economically viable processes to convert biomass into value-added products such as biofuels and biobased chemicals is of high relevance. Fast pyrolysis technology is a well-known primary biomass conversion technology,[1] able to liquefy biomass in a relatively inexpensive and simple manner, yielding up to 70 wt %[2] of a so-called pyrolysis liquid. This liquid is a microemulsion in which the continuous phase is an aqueous solution of cellulose and hemicellulose decomposition products that stabilizes a discontinuous phase of lignin oligomers, among others, by mechanisms such as hydrogen bonding.[3] Due to the high oxygen content, acidity, limited thermal stability, and low heating value, pyrolysis liquids need further upgrading to improve product properties.

Pyrolysis liquids can be either upgraded as a whole or after fractionation. Fractionation is possible by water addition, leading to a water phase enriched in sugar derivatives (sugar fraction) and an organic phase with the lignin-derived fragments (pyrolytic lignin).[4] The aqueous phase contains mostly low molecular weight C_6 anhydrosugars and other polar components, which can be used as feedstock in relatively well-developed processes (e.g., fermentation,[5,6] hydrogenation,[7] hydrogenolysis,[8] hydrolysis[9]), as well as oligomeric sugars. On the other hand, the pyrolytic lignin (PL) fraction consists of a complex three-dimensional phenolic network with lower acidity and oxygen content. The valorization of PL is less straightforward due to its structural features and high chemical heterogeneity, often needing harsh reaction conditions for subsequent conversions[10] in comparison to the sugar-based fraction.

Received: September 22, 2018
Revised: February 5, 2019
Published: February 12, 2019

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

In detail, pyrolytic lignin is a complex mixture of oligomers with HGS (hydroxyphenyl, guaiacyl, and syringyl) building blocks connected via a number of linkages.[11] ^{13}C NMR studies have indicated that these linkages differ significantly from those in native lignin and are predominantly in the form of interunit C−C linkages.[11] A recent work[12] further elucidated the structure of PL by means of advanced NMR techniques such as HSQC and HMBC NMR. The proposed structure contains mainly biphenyl, diaryl−ether, and diaryl−methine linkages, in combination with HGS units and alkane, alkene, and carbonyl groups (see Figure 1).

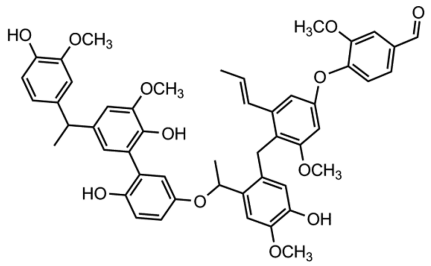


Figure 1. Main aromatic units and linkages in a guaiacyl-based pyrolytic lignin. Adapted from ref 12.

Several upgrading strategies for native lignin have been explored in the literature,[13] from which oxidation stands as a promising approach. The structure of lignin is particularly prone to oxidation due to the presence of aromatic rings with high electronic density. A number of studies involve (bio)-catalyzed reactions aiming for the selective depolymerization of both native and technical lignins (e.g., Kraft, sulphite, and organosolv).[14−18] Interesting products from lignin oxidation processes are aromatic compounds and dicarboxylic acids (DCAs), the latter being valuable in various applications in the polymer and food industries.[19−22] A well-known example of an important DCA is adipic acid, which is mainly used (85%) for the manufacture of nylon-6.6.[23] The global production of adipic acid was estimated at 3.3 million tons in 2016,[24] and the production is expected to grow 4.1% annually.

DCAs are currently obtained from fossil resources, and green synthesis routes are highly attractive. Previous investigations showed that technical lignins such as steam-exploded, alkali, and organosolv could be oxidized into DCAs in good yields and selectivities.[25,26] Oxidation was also successfully applied as a pretreatment for stabilizing pyrolysis liquids.[27−31]

Ozone is considered an attractive oxidizing agent among the various oxidation options available. It can be easily generated from oxygen using well-established technology available on a number of scales. A previous study on the ozonation of lignin derived from a commercial alkali feedstock resulted in the formation of a product mixture that was shown to be suitable for use as a fuel additive.[32]

In contrast to lignin oxidation, the oxidation of PL remains relatively unexplored. Recently, the use of $H_5PMo_{10}V_2O_{40}$ as a catalyst for the oxidation of PL derived from pyrolysis of rice husk was reported, and a range of aromatics were identified in the product mixture.[33] We here report the ozonation of a pine-derived PL with the objective to depolymerize it into lower molecular weight aromatics and/or bifunctional organic acids and esters. In addition, model component studies with representative low molecular weight compounds and a well-defined guaiacyl-based biosynthetic lignin were performed to rationalize the results and to propose a reaction network.

MATERIALS AND METHODS

Chemicals. Pine-derived PL was supplied by the Biomass Technology Group (BTG, Enschede, The Netherlands). The water-insoluble PL fraction was obtained by water extraction of standard pyrolysis oil. The pyrolysis oil was produced at 550 °C in a rotating cone reactor[34] (pilot plant with capacity of 3 ton of pyrolysis oil/day). Relevant properties are presented in Table 1, and further characterization details are provided in the Supporting Information. An overview of the suppliers of chemicals and their purity is given in the Supporting Information. All chemicals were used as received. Ozone was produced using a commercial ozone generator (Model 501 from FISCHER Labor and Verfahrenstechnik) with oxygen gas as the feed.

Table 1. Relevant Properties of the Pyrolytic Lignin Used in This Study

property	value (wt %)
water content	4.68
sugar derivatives, dry basis	4.98[a]
elemental composition, dry basis	
C	65.9
H	6.48
O	27.5
N	<0.01

[a]Mainly levoglucosan and glycoaldehyde, identified and quantified by HPLC.

Ozonation of Pyrolytic Lignin and Model Compounds. PL ozonation experiments were conducted in a batch reactor (see Supporting Information Figure S1 for a schematic representation). The reactor was loaded with a solution of PL (20 g) dissolved in methanol (40 g). The reactor content was cooled to 0 °C using an ice bath, and ozone (3 g/h) was introduced in the reactor using a dip tube. Information regarding ozone concentration was provided by the ozone generator manufacturer and confirmed through a titration method detailed in the Supporting Information. The ozonation was performed at atmospheric pressure, and reaction times varying from 15 to 240 min were evaluated. At the end of each experiment, the ozone flow was stopped and replaced by nitrogen gas for 15 min to remove any residual ozone. Methanol was removed in a rotary evaporator at 300 mbar and 45 °C for 1 h, and the resulting liquid product was analyzed in detail. Yields were determined on the basis of the ozonated oil after methanol removal (i.e., isolated product).

A small-scale PL ozonation experiment with labeled ^{13}C-methanol was performed in a 4 mL vial, in which around 200 mg of PL was diluted in 500 mg of ^{13}C-methanol. The solution was exposed to ozone for 2 min. The ozone input and reaction conditions were the same as for the large-scale experiments described above. ^{13}C-methanol was removed by evaporation (300 mbar and 45 °C for 1 h), and the resulting liquid product was analyzed in detail. An identical experiment was performed with nonlabeled methanol to serve as control.

Ozonation experiments of model compounds were performed in a similar fashion but on smaller scale (50 mL). The model component (1 mmol) was diluted in methanol (20 g). Bicyclohexyl was used as the internal standard. Reaction conditions and ozone input were the same as for experiments with PL, and the total reaction time was fixed to 20 min for each experiment. Samples were taken from the solution every 2 min and analyzed by GC/MS-FID.

Ozonation experiments of the biosynthetic lignin oligomer (preparation details are reported elsewhere,[35] and the proposed structure is given in Figure S2) were performed with the lignin (30 mg) dissolved in methanol-d_4 (600 mg). The mixture was subjected to a 1 min reaction under the same conditions and ozone input as for

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

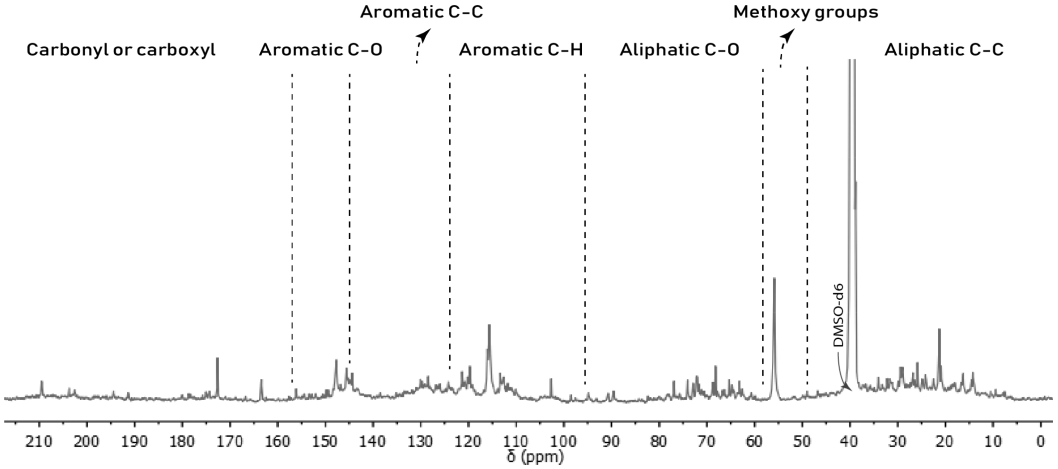


Figure 2. ^{13}C NMR spectrum of the PL used in this study (DMSO-d_6).

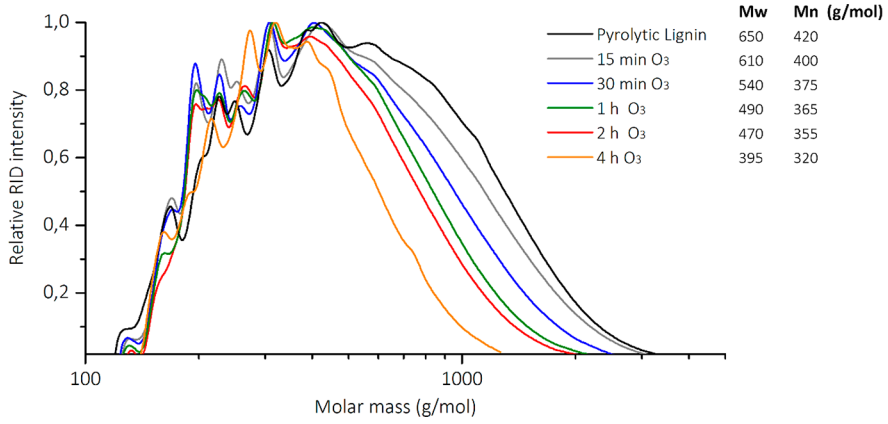


Figure 3. Molecular weight distribution of the isolated products versus ozonation time (GPC).

the experiments with model components. A HSQC NMR spectrum was taken directly after the reaction.

Product Analyses. GPC analyses were performed using an Agilent HPLC 1100 system equipped with a refractive index detector and three columns in series of MIXED type E (length 300 mm, i.d. 7.5 mm). Polystyrene was used as a calibration standard. Details are given in the Supporting Information.

^{13}C NMR spectra were recorded by a Varian Unity Plus (400 MHz) using a 90° pulse and an inverse-gated decoupling sequence with relaxation delay of 10 s, sweep width of 225 ppm, and 1024 scans. Samples were dissolved in DMSO-d_6 (50 wt %) prior to analysis. Details are given in the Supporting Information.

Heteronuclear single quantum coherence (HSQC) 2D-NMR spectra were recorded by a Varian Unity Plus (400 MHz) with the following parameters: 11 ppm sweep width in F_2 (1H), 220 ppm sweep width in F_1 (^{13}C), 8 scans, and 1024 increments.

The high-performance liquid chromatography (HPLC) analytical device consisted of an Agilent 1200 pump, a Bio-Rad organic acids column Aminex HPX-87H, a Waters 410 differential refractive index detector, and a UV detector. Details are given in the Supporting Information.

Gas chromatography/mass spectrometry (GC/MS-FID) analyses were performed on a Hewlett-Packard 5890 gas chromatograph equipped with a RTX-1701 capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness) and a Quadrupole Hewlett-Packard 6890 MSD selective detector attached. Details are given in the Supporting Information.

RESULTS AND DISCUSSION

Composition of the PL. The PL used in the study was obtained from the fractionation of a fast pyrolysis liquid derived from pinewood. The lignin structure of pinewood, a typical example of a softwood, consists mainly of guaiacyl (G) units and trace amounts of *p*-hydroxyphenyl (H) units.[36] Analyses show that the PL contains still 4.68 wt % of water, as well as some sugar derivatives (4.98 wt %). The latter were identified mainly as levoglucosan and glycoaldehyde (HPLC). The molecular weight of the PL was determined by GPC and found to be 650 g/mol (M_w), which is indeed by far lower than found for a typical organosolv lignin (Alcell) using the same analytical equipment and method (i.e., M_w of 1720 g/mol[37]). GCxGC-FID analysis shows the presence of mainly phenolics and some small acids, ketones, and aldehydes, mainly levoglucosan and glycolaldehyde (confirmed by HPLC). The total amount of GC detectables was only 11 wt %, indicating that the majority of the components are oligomeric in nature and not sufficiently volatile to be detected with GC. This is also in line with the GPC data. GC/MS-FID confirms the GCxGC analysis and shows the presence of guaiacol and para-substituted alkylguaiacols, as well as levoglucosan and glycolaldehyde.

The ^{13}C NMR spectrum of PL shows a large number of peaks belonging to different functional groups. By integrating the seven main areas belonging to carbon atoms in different structural environments reported in the literature[38] (see Figure

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

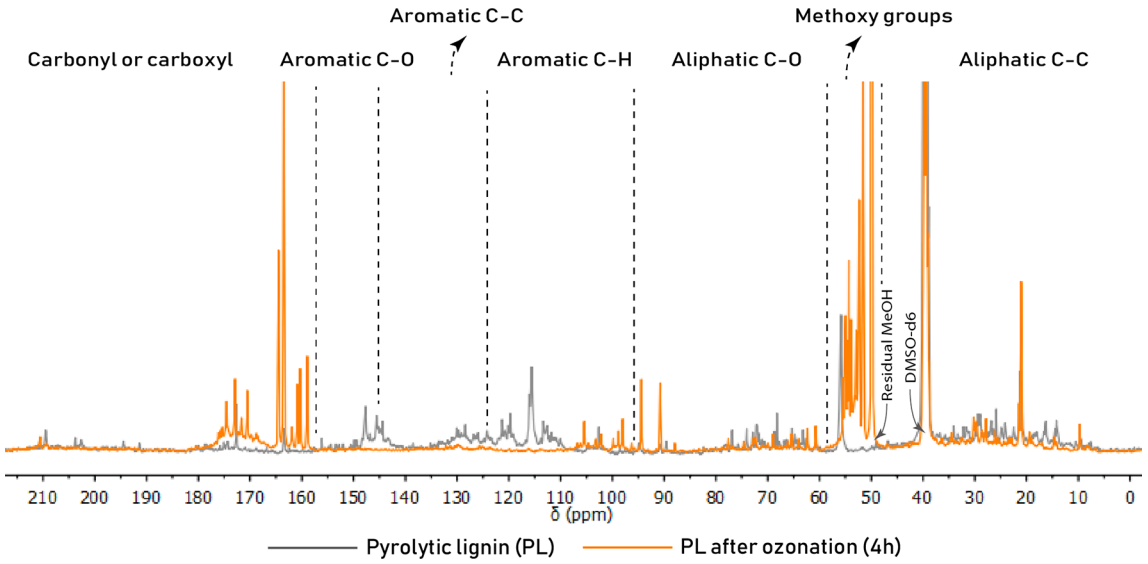


Figure 4. ^{13}C NMR spectra of the PL feed and the isolated product after 4 h ozonation (DMSO-d_6).

2), a rough estimate could be obtained for relevant chemical groups (percentages based on the integrated peak areas): 31.5% of aliphatic C−C bonds, 5.2% of methoxy groups, 6.3% of aliphatic C−O bonds, 53% of aromatics (total of C−H, C−C, and C−O aromatic bonds), and 4% of carbonyl and carboxyl groups.

Ozonation Experiments with PL. Initial ozonation experiments were performed using the PL dissolved in methanol at 0 °C and atmospheric pressure at different batch times. Significant visual changes were observed upon ozonation, and after extended reaction times the original dark brown color of the PL feed changed to vivid orange, without the occurrence of phase separation. In addition, after methanol removal, the products became increasingly soluble in water (i.e., up to 85 wt %, see Figure S4).

Mass balance calculations were performed for an experiment at a slightly larger scale. A mass gain of 37 wt % was calculated, likely due to the incorporation of oxygen (from ozone) and methanol in the products (mostly as methoxy groups of esters and acetals/ketals, vide infra). However, mass losses by the formation of gas phase components like CO_2 are also possible due to overoxidation of products. As CO_2 formation could not be quantified accurately, a full mass and carbon balance could not be determined. The isolated products were analyzed in great detail to obtain qualitative and quantitative information. Two methods can be distinguished: (i) those giving simultaneous information on the monomeric and oligomeric fraction (GPC, NMR) and (ii) those giving information on the monomeric fraction (GC, HPLC).

Simultaneous Analyses of Monomeric and Oligomeric Products. The molecular weight distributions of the PL feed and isolated products obtained after different ozonation times were determined by GPC (Figure 3). The average molecular weight decreased up to 40% after 4 h ozonation, indicating further breakdown of the PL structure upon extended ozonation times.

The volatility of the samples was determined using TGA (see Supporting Information, Figure S5). The original PL shows a gradual weight loss in the temperature range between 100 and 450 °C, ultimately leading to about 30% of residue. The volatility of the ozonated samples is by far higher, and for instance for the 4 h ozonation product, weight loss already takes place to a significant extent below 100 °C. These findings support the GPC measurements that substantial reduction of molecular weight has occurred with the concomitant formation of low molecular weight, more volatile components.

^{13}C NMR spectra were acquired to obtain qualitative and quantitative information on the types of chemical functionalities present in the products (Figure 4). Significant differences were observed between the PL feed and the 4 h ozonated product. The signals in the aromatic region practically disappear, while new methoxy and carbonyl groups are formed. The majority of the aliphatic C−O signals are lowered, and some of the reminiscent ones are related to the presence of residual levoglucosan, which is relatively inert to ozone attack (vide infra). Although the long relaxation times associated with the carbon nuclei impede absolute integration of the peaks, the relative ratios are still instructive. The aromatic/ether ratio (i.e., sum of aromatic C−O, C−C, and C−H areas divided by the sum of methoxy groups and aliphatic C−O areas) decreased from 4.6 to 0.1 due to the drastic loss of aromaticity and formation of new methoxy groups. The aromatic/carbonyl ratio decreased from 13.2 to 0.1, as carbonyl groups were also extensively formed during ozonation together with the reduction of aromatics. All findings indicate that the aromatic rings are very reactive toward ozone and are transformed to other (nonaromatic) structures. Additional analyses such as FTIR and determination of the water content ratified the loss of aromaticity and showed a gradual increase in water content (see Figures S6 and S7). In addition, CAN analysis and acid titrations showed an increase in both the carbonyl and carboxylic acid groups upon exposure to ozone (see Figures S8 and S9). These results suggest that the peaks in the carbonyl regions are indeed associated with the formation of carboxylic acids and their respective methyl esters. The latter is also confirmed by the extensive formation of new methoxy groups (50−60 ppm range).

Further insights in the product composition after ozonation were obtained by 2D-NMR HSQC measurements. For the PL feed, four main peak areas are observed: aliphatics (alkyl side chains), methoxy groups (from the guaiacyl units), oxygenated

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

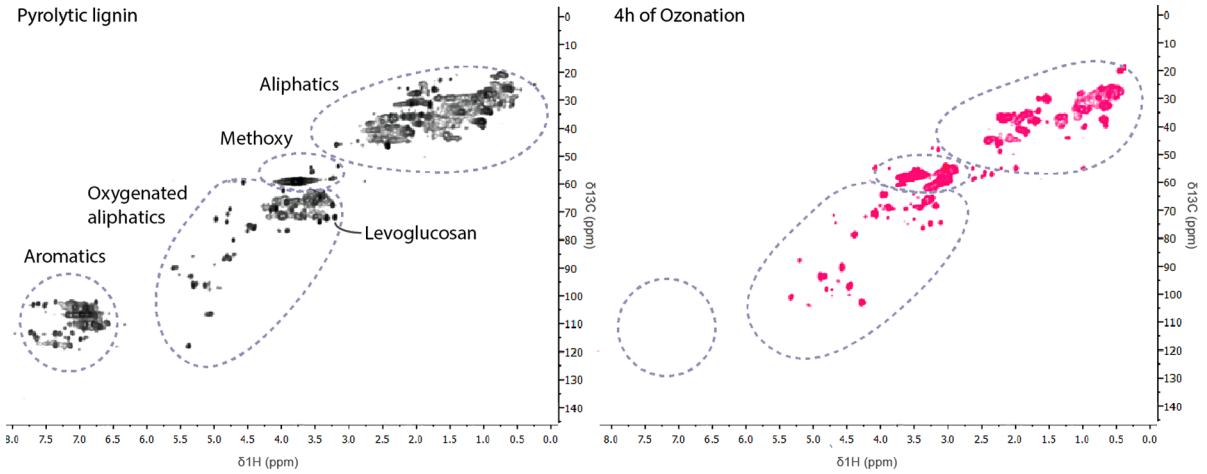


Figure 5. 2D-NMR HSQC spectra of the PL feed and the isolated product after 4 h ozonation (DMSO-d_6).

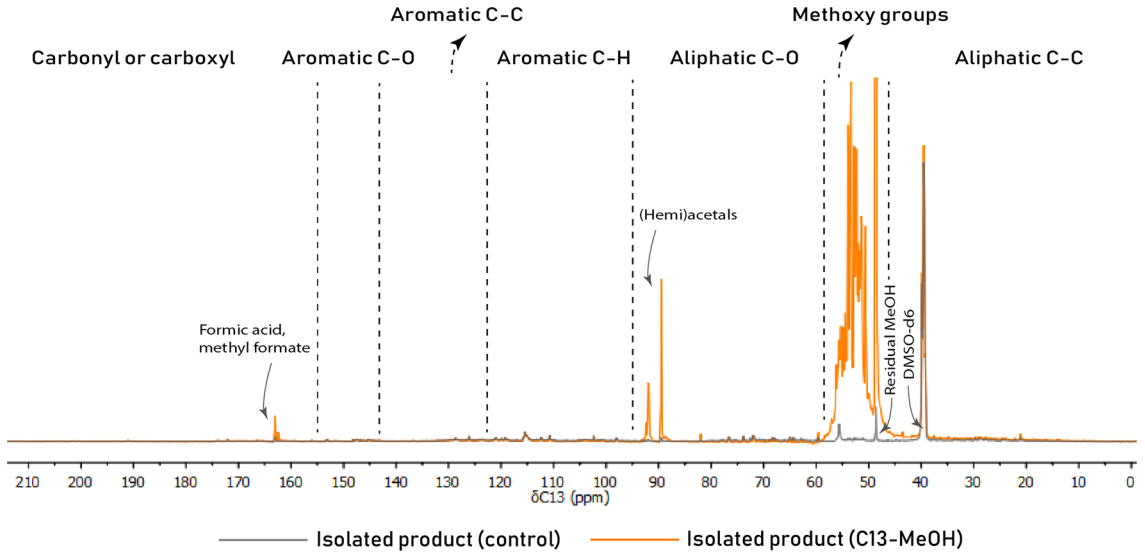


Figure 6. ^{13}C NMR spectra of the ozonated PL with methanol (control experiment) and ^{13}C-methanol (DMSO-d_6).

aliphatics (residual sugars and ether bonds existent within the aromatic network), and aromatics (mostly guaiacyl units due to the biomass source used), see Figure 5 for details. Quaternary carbon atoms are not detectable with this technique, and as such the presence of carbonyls in the form of carboxylic acid/ester groups cannot be confirmed through this analysis.

Upon exposure to ozone, the peaks in the aromatic region fully disappear, indicating the occurrence of ring-opening reactions. Significant changes are also observed in the methoxy area. The original signals, arising from guaiacol units in the PL feed, have disappeared, and new methoxy groups are present, likely from methyl esters. Several new signals are also observed in the oxygenated aliphatics region. In addition, peaks associated with levoglucosan are still present in the ozonated samples, indicating that it is relatively inert (also supported by model component studies, vide infra).

Product Characterization: Methanol Incorporation in the Products. A small-scale ozonation experiment with labeled ^{13}C-methanol was performed to evaluate qualitatively and quantitatively its incorporation within the ozonated product mixture. Figure 6 shows the ^{13}C NMR spectra of the product mixture obtained from the reaction performed in ^{13}C-methanol overlaid with a control experiment at the same scale and conditions using nonlabeled methanol. The results clearly show a ^{13}C-enriched area, particularly in the range of the methoxy signals. This implies that methanol is incorporated mostly as methoxy groups in esters or ketal/acetal moieties within the product mixture. In addition, products derived from the oxidation of methanol itself were observed in minor amounts (i.e., ^{13}C-formic acid, ^{13}C-formaldehyde) including the subsequent esterification of ^{13}C-formic acid (i.e., methyl formate) and acetals/hemiacetals of ^{13}C-formaldehyde (see Figures S10 and S11 for HSQC and HMBC NMR spectra). Thus, methanol oxidation only occurs to a minor extent. Integration of the methoxy area (50−60 ppm range, DMSO-d_6 peak as reference) indicates that the isolated product contains 19.5 wt % of methoxy groups originating from the solvent (methanol). The amount of compounds from methanol oxidation was estimated to be 1.8 wt % (based on isolated product).

Product Characterization: Low Molecular Weight Fraction. To identify and quantify the amounts of low molecular weight carboxylic acids and methyl esters thereof, the samples were diluted with water, leading to the formation of two liquid phases, the exact amounts of both phases being a

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

function of the ozonation time (see Supporting Information, Figure S4). The water-soluble fractions were further analyzed by HPLC. The analysis was performed under acidic conditions in water, leading to (partial) hydrolysis of the esters. As such, the individual amounts of esters and acids in the product cannot be assessed. The total yield of (di)acids was up to 39.3 wt % (based on isolated product oil, see Figure 7) after 4 h reaction, evidencing the formation of substantial amounts of organic acids/esters by the reaction of the PL with ozone and methanol.

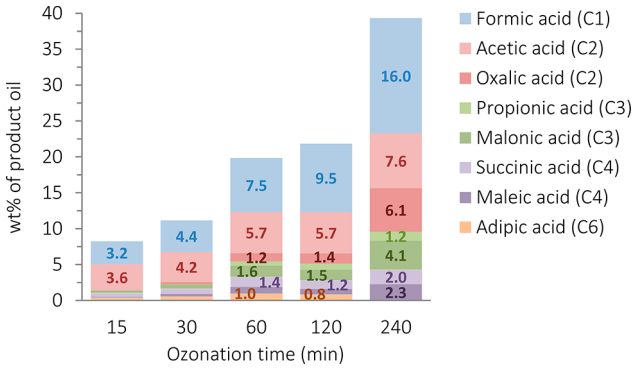


Figure 7. Acid distribution versus ozonation time (HPLC, based on isolated product oil).

GC-MS analysis of the product confirms the formation of the esters upon ozonation (up to 80 area % of the GC-detectables, respectively, see Figure 8). Furthermore, some acetals are present, as well as levoglucosan. The long-chain aliphatic esters are likely derived from fatty components initially present in the biomass. It is worth mentioning that most of the produced esters and dicarboxylic acids are thermally unstable or nondetectable by FID (i.e., formic acid); thus, the identification via the used GC method is limited for the ozonated oils.

Product Characterization: High Molecular Weight Fraction. To obtain qualitative insights with respect to the chemical structure of the higher molecular weight fraction after ozonation, the low molecular weight fraction was removed by vacuum evaporation (100 mbar and 75 °C, 1 h). GC analysis showed only minor peaks, indicating that the residual amounts of low volatile, GC detectables are low. Furthermore, TGA and GPC further confirmed the oligomeric character of this fraction (see Supporting Information, Figures S12−S14). Subsequently, it was extracted with D_2O, and the extract was analyzed using 2D-NMR HSQC (see Supporting Information, Figure S15). The spectrum shows clear resonances in the aliphatic, methoxy, and oxygenated aliphatics region, whereas peaks in the aromatic region are absent. It suggests that the oligomeric fraction is composed of an aliphatic network with sides chains containing, among others, ester groups.

Product Overview. Based on product analysis of the ozonated PLs, we can conclude that the original highly aromatic network is substantially depolymerized (via ring-opening pathways and subsequent oxidative cleavage of alkenes) upon the ozone treatment, forming mainly low molecular weight mono and diacids/esters and some acetals, as well as an oligomeric fraction mainly comprised of a highly oxygenated aliphatic backbone (Figure 9). Based on the quantification of acids (vide supra) and GCxGC analyses (see Supporting Information, Figure S16), the low molecular weight fraction corresponded to around 45 wt % of the isolated product oil after a 4 h ozonation.

Ozonation of Model Compounds. To rationalize the product composition after PL ozonation, the ozonation of representative model components was investigated. Thirteen molecules were selected belonging to three discrete groups: (i) monomeric phenolic compounds typically present in pyrolytic lignins; (ii) dimeric phenolic components with different linkages between the aromatic units (i.e., benzophenone, diphenyl oxide, 1,2-diphenylethane, β-O-4 dimers); (iii) levoglucosan, typically present as a byproduct in PLs and derived from the (hemi)-cellulose fraction in the original biomass PL (vide supra).

Figures 10a and 10b show that the reactivity trends varied substantially in both monomeric and dimeric groups of model compounds. Due to the relatively low electronic density, purely aromatic and saturated aliphatic carbon bonds are known to be stable against ozone attack.[39] The results observed in Figure 10a suggest that ether, carbonyl, and β-O-4 linkages in nonsubstituted aromatic dimers are also resistant to ozonation.

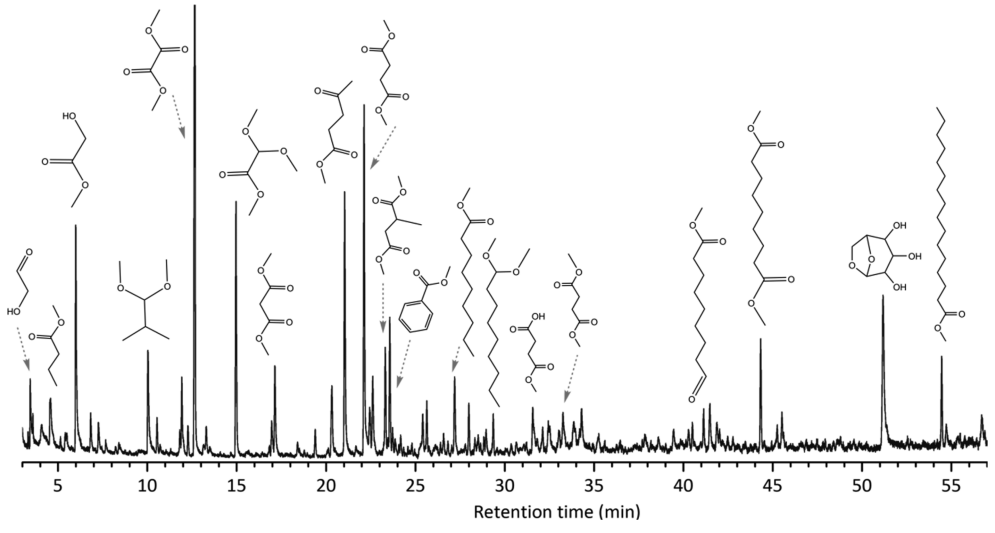


Figure 8. GC-MS spectrum for the isolated product obtained after 4 h ozonation of PL.

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

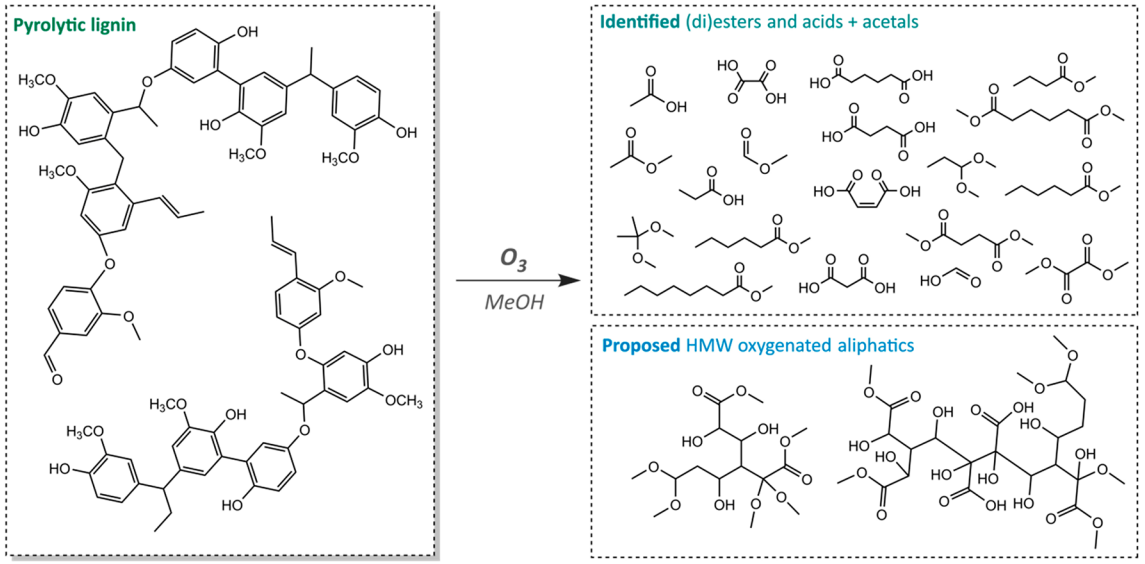


Figure 9. Molecular representation of the ozonation reaction of PL.

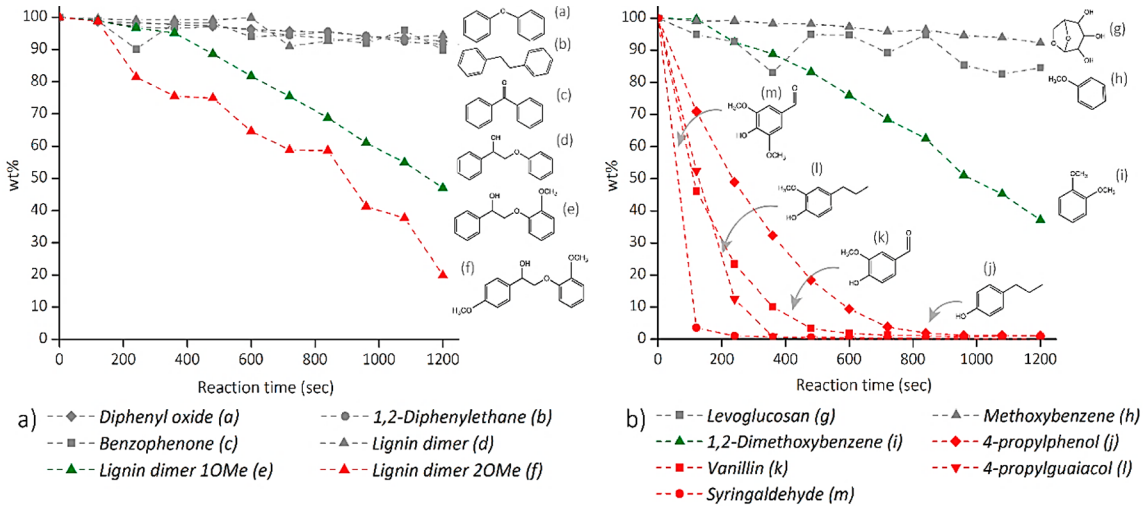


Figure 10. Reactivity trends of the (a) dimeric and (b) monomeric model compounds during ozonation.

For instance, the reactivity gradually increased for the methoxylated and dimethoxylated β-O-4 dimers (i.e., molecules e and f), which is envisioned due to the electron-donating character of the methoxy group.[40,41]

In Figure 10b the low reactivity of levoglucosan (g) is shown, and this result is in accordance with the ozonation of pyrolytic lignin, in which levoglucosan can be identified even after long reaction times. A similar trend as seen for the dimeric models is also observed for methoxybenzene (h) and dimethoxybenzene (i), i.e., the latter presenting higher reactivity. Compounds containing a phenolic group (i.e., molecules j, k, l, and m) were converted significantly faster compared to the other model compounds. In addition, the presence of a methoxy substituent increased guaiacol (l) reactivity in comparison to phenol (j), while the aldehyde group, which is electron-withdrawing, caused vanillin (k) to be slightly less reactive than guaiacol (l). The most reactive model compound was syringaldehyde. This reactivity pattern syringyl > guaiacyl >4-hydroxyphenyl for ozonation is in line with literature data.[42]

The reactivity of ozone clearly depends on the aromatic substituents, which can either donate or withdraw electronic density from the system. Overall, the phenolic functionality had the strongest effect on reactivity, followed by the methoxy substituent. From the model compounds results, it can be inferred that the high reactivity of pine-derived pyrolytic lignin toward ozone is a direct consequence of its largely methoxylated phenolic structure, rich in guaiacyl (G) units.[36] Further qualitative analyses of the ozonated model compound solutions showed the presence of various (di)acids (see Supporting Information, Figures S17 and S18 for 4-propylguaiacol). These results are in line with the ones observed for PL ozonation (vide supra).

Ozonation of a Biosynthetic Lignin Oligomer. The ozonation of a lignin oligomer with well-defined phenolic structure (obtained through a biocatalytic cascade starting from eugenol,[35] see Supporting Information, Figure S2) was also investigated to substantiate the model component studies, and to gain insights in the stability of the various linkages upon treatment with ozone. A small-scale ozonation experiment was performed with the biosynthetic lignin oligomer diluted in

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

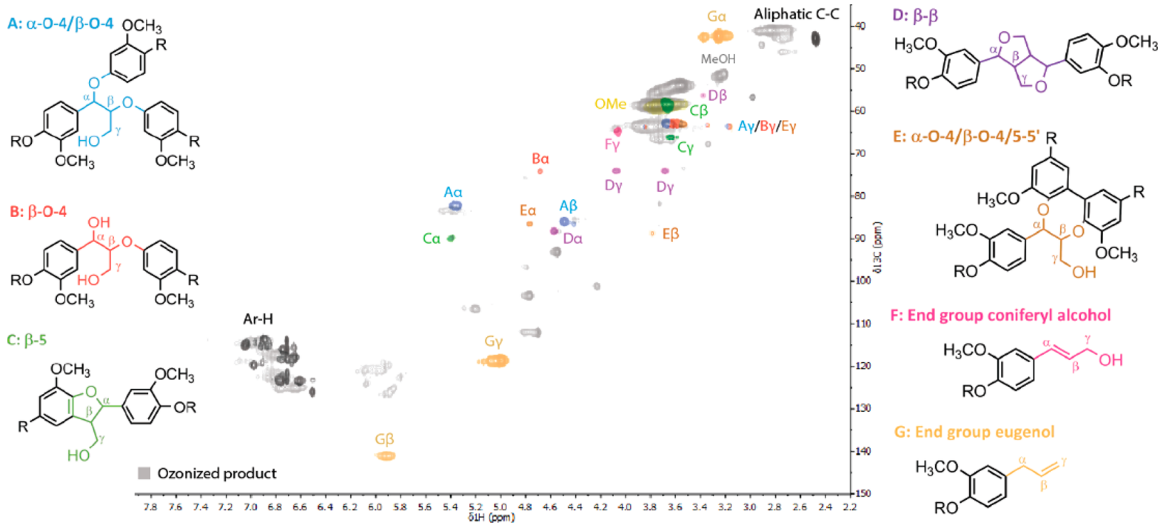


Figure 11. 2D-NMR HSQC spectra of the lignin oligomer before and after ozonation (methanol-d_4).

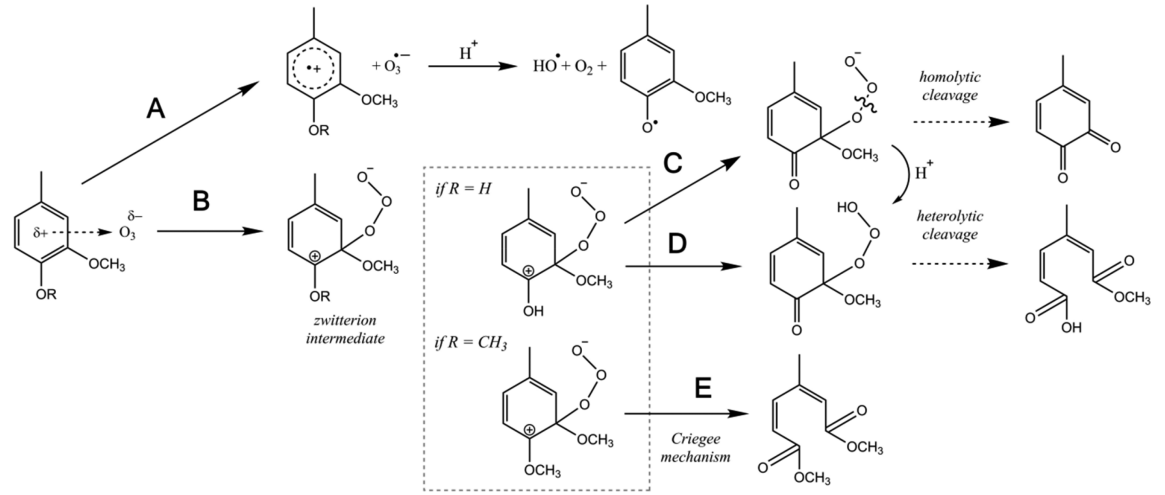


Figure 12. Proposed mechanisms for the initial reaction between ozone and an aromatic lignin-like structure (A, B) and further reaction pathways (C, D, E). Adapted from ref 47.

methanol-d_4, and NMR HSQC spectra were directly acquired to access the main changes in the structure. Figure 11 shows the NMR spectra before and after ozonation, highlighting the main types of bonds initially present and their respective signals.

The biosynthetic lignin contains linkages usually identified in (guaiacyl-based) lignins[12,43] such as β-O-4, β–β, β-5, and aliphatic C–C double bonds. The NMR spectrum of the ozonated product showed various new signals related to methoxy groups and oxygenated aliphatics.

The rapid disappearance of the strong eugenol and coniferyl end group signals (F and G) is likely due to the high reactivity of ozone toward the oxidation of aliphatic C=C bonds in comparison with aromatic C=C bonds.[39] However, substantial cleavage of the aromatic rings also takes place during ozonation as is evident from the reduction of associated resonances (structures A, B) or complete disappearance (structures C, D, E). From the model compounds study discussed previously, it is known that hydroxy substituents have a strong effect on the reactivity of an aromatic structure toward ring-opening reactions. As such, the presence of an −OH or −OR group in the aromatic C1 will lead to either readily reactive or relatively more stable structures.

Based on the ozonation of PL, peaks associated with the formation of low molecular weight esters as well as oligomeric structures with aliphatic backbone and oxygenated side chains are anticipated. Indeed, new signals are found in the area associated with the methoxy groups of the esters (3.6 ppm δ^1H; 55 ppm δ^{13}C), while the signals in the δ^{13}C region of 90–115 ppm are indicative of new ether bonds, likely formed in ring-opening reactions. The new signals in the δ^{13}C region of 120–130 ppm are related to highly oxygenated groups such as acetals.

MECHANISTIC IMPLICATIONS

The oxidation of multifunctionalized networks such as lignin is a complex multistep process, usually involving radical and molecular mechanisms[44] and several intermediates. Further recombination reactions substantially expand the product slate, and the presence of radicals favors repolymerization.[33,45,46]

In line with these general findings for the oxidation of lignin, ozone is reported[47] to react with the lignin-derived structures via a sequence involving a number of different types of

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755–4765

reactions. The first step involves reaction of ozone with the aromatic ring, via either radical or 1,3-dipolar additions (A, B), followed by further reaction pathways involving homolytic or heterolytic cleavages (C, D, E),[47] see Figure 12 for details.

Pathway A involves electron transfer and results in an aromatic cation radical along with an ozonide radical that further decomposes into oxygen, a hydroxyl radical, and a phenoxyl radical. The radical intermediates are very unstable and may lead to repolymerization.[33,45,46] Mechanism B occurs via an electrophilic ozone attack preferentially on the oxygen-substituted carbon of the aromatic ring. The resulting zwitterion intermediate subsequently reacts via different routes depending on the reaction medium and reactant nature. On guaiacolic structures (i.e., R = H) the hydroxyl substituent can be immediately deprotonated, forming a keto-function that can further yield a quinone product via homolytic cleavage (route C) or react via heterolytic cleavage (route D) yielding ring-opening products such as muconic acid and its derivatives. Even though routes C and D can occur simultaneously, it is reported[47] that acidic conditions promote the later one. Nonphenolic structures (i.e., R = CH_3) will behave differently since deprotonation of the zwitterion is not possible. Under acidic conditions, heterolytic ozonation (route E) is expected to be predominant, forming ring-opening products as well.

All PL ozonation experiments reported here were performed under acidic conditions, which are known to suppress radical pathways,[47] and methanol is also reported as an efficient radical quencher.[48] Therefore, it is expected that repolymerization does not occur extensively, in line with the experimental data (GPC). The ozonation of PL, as well as the model components, demonstrates that depolymerization occurs through mechanisms involving phenolic moieties, generating a range of low molecular weight DCAs and methyl (di)esters along with higher molecular weight molecules consisting of a highly oxygenated aliphatic backbone (Figure 9). Pyrolytic lignin consists mostly of ortho-methoxylated, para-alkylated phenolic structures, and the electron-releasing character of these groups makes the structure highly prone to ozone attack.[14] Ring-opening mechanisms can be recognized when observing the product spectra of PL ozonation, and its guaiacyl-based structure favors particularly the pathway D. Since the immediate ring-opening products are very susceptible to further ozonation (due to the aliphatic C−C double bonds), degradation is expected to happen, yielding the range of smaller molecules detected by HPLC (e.g., oxalic, maleic, malonic acids).

In addition to DCAs, a number of methyl esters and acetals were identified by GC/MS analysis. Three pathways are proposed for the formation of esters (see Supporting Information, Figure S19), from which the most likely one under the applied conditions involves the esterification of initially formed organic acids with methanol. In addition, reactions of carbonylated products (i.e., ketones and aldehydes) with methanol lead to the formation of ketals and acetals.[49]

CONCLUSIONS

Ozone treatment of PLs results in extensive depolymerization, generating up to 45 wt % of low molecular weight dicarboxylic acids/esters, along with oligomeric structures consisting of a highly oxygenated aliphatic backbone. Depolymerization occurs mostly via the heterolytic cleavage of aromatic rings and cleavage of interaromatic bonds with high electronic density. Secondary oxidation and esterification reactions broaden the product spectra. The presence of -hydroxy and -methoxy substituents on the aromatic network, i.e., dominant side groups within the initial structure, were shown to enhance reactivity for ozone.

These findings show that ozonation is a straightforward, clean, and inexpensive depolymerization route for PLs. The reaction is performed at mild conditions and does not require a catalyst. Furthermore, it uses simple solvents such as methanol, which can be biobased and recovered for reuse. The low molecular weight fraction contains value-added DCAs and platform molecules such as adipic, oxalic, and succinic acid, as well as ester mixtures interesting for applications related to fuels, additives, polymers, and resins. Further process optimization and the development of efficient separation technology will be required and is currently the focus of our research activities in this field.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.8b04856.

Detailed characterization of pyrolytic lignin, methods, and results of additional analyses of the ozonated oils (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: h.j.heeres@rug.nl. Tel.: +31 50 3634174.

ORCID

Peter J. Deuss: 0000-0002-2254-2500

Hero J. Heeres: 0000-0002-1249-543X

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support from the Science without Borders program (Brazil) for the Ph.D. project of M.B.F. is gratefully acknowledged. We also thank Erwin Wilbers, Marcel de Vries, Léon Rohrbach, Jan Henk Marsman, and Anne Appeldoorn for technical support and BTG. for supplying the pyrolytic lignin. Mohamed Habib is gratefully acknowledged for providing the biosynthetic lignin.

REFERENCES

(1) Faaij, A. Modern biomass conversion technologies. *Mitigation Adapt. Strat. Glob Change* **2006**, *11*, 343−375.

(2) Bridgwater, A. V. Renewable fuels and chemicals by thermal processing of biomass. *Chem. Eng. J.* **2003**, *91* (2−3), 87−102.

(3) Meier, D.; van de Beld, B.; Bridgwater, A. V.; Elliott, D. C.; Oasmaa, A.; Preto, F. State-of-the-art of fast pyrolysis in IEA bioenergy member countries. *Renewable Sustainable Energy Rev.* **2013**, *20*, 619−641.

(4) Oasmaa, A.; Kuoppala, E.; Solantausta, Y. Fast pyrolysis of forestry residue. 2. Physicochemical composition of product liquid. *Energy Fuels* **2003**, *17* (2), 433−443.

(5) Wang, H.; Livingston, D.; Srinivasan, R.; Li, Q.; Steele, P.; Yu, F. Detoxification and fermentation of pyrolytic sugar for ethanol production. *Appl. Biochem. Biotechnol.* **2012**, *168* (6), 1568−1583.

(6) Rover, M. R.; Johnston, P. A.; Jin, T.; Smith, R. G.; Brown, R. C.; Jarboe, L. Production of clean pyrolytic sugars for fermentation. *ChemSusChem* **2014**, *7* (6), 1662−1668.

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

(7) Yin, W.; Venderbosch, R. H.; Alekseeva, M. V.; Figueirêdo, M. B.; Heeres, H.; Khromova, S. A.; Yakovlev, V. A.; Cannilla, C.; Bonura, G.; Frusteri, F.; Heeres, H. J. Hydrotreatment of the carbohydrate-rich fraction of pyrolysis liquids using bimetallic Ni based catalyst: Catalyst activity and product property relations. *Fuel Process. Technol.* **2018**, *169*, 258−268.
(8) Ruppert, A. M.; Weinberg, K.; Palkovits, R. Hydrogenolysis goes bio: from carbohydrates and sugar alcohols to platform chemicals. *Angew. Chem., Int. Ed.* **2012**, *51* (11), 2564−2601.
(9) Bennett, N. M.; Helle, S. S.; Duff, S. J. Extraction and hydrolysis of levoglucosan from pyrolysis oil. *Bioresour. Technol.* **2009**, *100* (23), 6059−6063.
(10) Kloekhorst, A.; Wildschut, J.; Heeres, H. J. Catalytic hydrotreatment of pyrolytic lignins to give alkylphenolics and aromatics using a supported Ru catalyst. *Catal. Sci. Technol.* **2014**, *4* (8), 2367−2377.
(11) Scholze, B.; Hanser, C.; Meier, D. Characterization of the water-insoluble fraction from fast pyrolysis liquids (pyrolytic lignin): Part II. GPC, carbonyl groups, and 13C-NMR. *J. Anal. Appl. Pyrolysis* **2001**, *58*−*59*, 387−400.
(12) McClelland, D. J.; Motagamwala, A. H.; Li, Y.; Rover, M. R.; Wittrig, A. M.; Wu, C.; Buchanan, J. S.; Brown, R. C.; Ralph, J.; Dumesic, J. A.; Huber, G. W. Functionality and molecular weight distribution of red oak lignin before and after pyrolysis and hydrogenation. *Green Chem.* **2017**, *19* (5), 1378−1389.
(13) Zakzeski, J.; Bruijnincx, P. C.; Jongerius, A. L.; Weckhuysen, B. M. The catalytic valorization of lignin for the production of renewable chemicals. *Chem. Rev.* **2010**, *110* (6), 3552−3599.
(14) Ma, R.; Xu, Y.; Zhang, X. Catalytic Oxidation of Biorefinery Lignin to Value-added Chemicals to Support Sustainable Biofuel Production. *ChemSusChem* **2015**, *8* (1), 24−51.
(15) Lange, H.; Decina, S.; Crestini, C. Oxidative upgrade of lignin − Recent routes reviewed. *Eur. Polym. J.* **2013**, *49*, 1151−1173.
(16) Crestini, C.; Crucianelli, M.; Orlandi, M.; Saladino, R. Oxidative strategies in lignin chemistry: A new environmental friendly approach for the functionalisation of lignin and lignocellulosic fibers. *Catal. Today* **2010**, *156* (1−2), 8−22.
(17) Hofrichter, M. Review: lignin conversion by manganese peroxidase (MnP). *Enzyme Microb. Technol.* **2002**, *30* (4), 454−466.
(18) Lancefield, C. S.; Ojo, O. S.; Tran, F.; Westwood, N. J. Isolation of Functionalized Phenolic Monomers through Selective Oxidation and C-O Bond Cleavage of the *β*-O-4 Linkages in Lignin. *Angew. Chem.* **2015**, *127* (1), 260−264.
(19) Zeikus, J.; Jain, M.; Elankovan, P. Biotechnology of succinic acid production and markets for derived industrial products. *Appl. Microbiol. Biotechnol.* **1999**, *51* (5), 545−552.
(20) Yadav, G.; Thathagar, M. Esterification of maleic acid with ethanol over cation-exchange resin catalysts. *React. Funct. Polym.* **2002**, *52* (2), 99−110.
(21) Lee, S. Y.; Hong, S. H.; Lee, S. H.; Park, S. J. Fermentative production of chemicals that can be used for polymer synthesis. *Macromol. Biosci.* **2004**, *4* (3), 157−164.
(22) Sato, K.; Aoki, M.; Noyori, R. A ″green″ route to adipic acid: Direct oxidation of cyclohexenes with 30% hydrogen peroxide. *Science* **1998**, *281* (5383), 1646−1647.
(23) Weissermel, K.; Arpe, H. Aromatics−Production and Conversion. In *Industrial Organic Chemistry*; Weissermel, K., Arpe, H., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: Weinhein, Germany, 2008; pp 313−336.
(24) Polen, T.; Spelberg, M.; Bott, M. Toward biotechnological production of adipic acid and precursors from biorenewables. *J. Biotechnol.* **2013**, *167* (2), 75−84.
(25) Ma, R.; Guo, M.; Zhang, X. Selective conversion of biorefinery lignin into dicarboxylic acids. *ChemSusChem* **2014**, *7* (2), 412−415.
(26) Hasegawa, I.; Inoue, Y.; Muranaka, Y.; Yasukawa, T.; Mae, K. Selective production of organic acids and depolymerization of lignin by hydrothermal oxidation with diluted hydrogen peroxide. *Energy Fuels* **2011**, *25* (2), 791−796.
(27) Tanneru, S. K.; Steele, P. H. Direct hydrocracking of oxidized bio-oil to hydrocarbons. *Fuel* **2015**, *154*, 268−274.
(28) Tanneru, S. K.; Steele, P. H. Pretreating bio-oil to increase yield and reduce char during hydrodeoxygenation to produce hydrocarbons. *Fuel* **2014**, *133*, 326−331.
(29) Luo, Y.; Guda, V. K.; Steele, P. H.; Wan, H. Hydrodeoxygenation of oxidized and hydrotreated bio-oils to hydrocarbons in fixed-bed continuous reactor. *BioResources* **2016**, *11* (2), 4415−4431.
(30) Luo, Y.; Guda, V. K.; Steele, P. H.; Mitchell, B.; Yu, F.; Hassan, E. B. Hydrodeoxygenation of oxidized distilled bio-oil for the production of gasoline fuel type. *Energy Convers. Manage.* **2016**, *112*, 319−327.
(31) Xu, J.; Jiang, J.; Dai, W.; Zhang, T.; Xu, Y. Bio-oil upgrading by means of ozone oxidation and esterification to remove water and to improve fuel characteristics. *Energy Fuels* **2011**, *25* (4), 1798−1801.
(32) Chuck, C. J.; Parker, H. J.; Jenkins, R. W.; Donnelly, J. Renewable biofuel additives from the ozonolysis of lignin. *Bioresour. Technol.* **2013**, *143*, 549−554.
(33) Zhao, Y.; Xu, Q.; Pan, T.; Zuo, Y.; Fu, Y.; Guo, Q. Depolymerization of lignin by catalytic oxidation with aqueous polyoxometalates. *Appl. Catal., A* **2013**, *467*, 504−508.
(34) Wagenaar, B.; Prins, W.; Van Swaaij, W. Pyrolysis of biomass in the rotating cone reactor: modelling and experimental justification. *Chem. Eng. Sci.* **1994**, *49* (24), 5109−5126.
(35) Habib, M.; Deuss, P.; Loncar, N.; Trajkovic, M.; Fraaije, M. A Biocatalytic One-Pot Approach for the Preparation of Lignin Oligomers Using an Oxidase/Peroxidase Cascade Enzyme System. *Adv. Synth. Catal.* **2017**, *359* (19), 3354−3361.
(36) Guo, H.; Zhang, B.; Qi, Z.; Li, C.; Ji, J.; Dai, T.; Wang, A.; Zhang, T. Valorization of lignin to simple phenolic compounds over tungsten carbide: Impact of lignin structure. *ChemSusChem* **2017**, *10* (3), 523−532.
(37) Kloekhorst, A.; Heeres, H. J. Catalytic hydrotreatment of Alcell lignin fractions using a Ru/C catalyst. *Catal. Sci. Technol.* **2016**, *6* (19), 7053−7067.
(38) Ben, H.; Ragauskas, A. J. NMR characterization of pyrolysis oils from kraft lignin. *Energy Fuels* **2011**, *25* (5), 2322−2332.
(39) Hoigné, J.; Bader, H. Rate constants of reactions of ozone with organic and inorganic compounds in water—I: non-dissociating organic compounds. *Water Res.* **1983**, *17* (2), 173−183.
(40) Charton, M. The application of the Hammett equation to ortho-substituted benzene reaction series. *Can. J. Chem.* **1960**, *38* (12), 2493−2499.
(41) Mvula, E.; Naumov, S.; von Sonntag, C. Ozonolysis of lignin models in aqueous solution: Anisole, 1, 2-dimethoxybenzene, 1, 4-dimethoxybenzene, and 1, 3, 5-trimethoxybenzene. *Environ. Sci. Technol.* **2009**, *43* (16), 6275−6282.
(42) Tanahashi, M.; Nakatsubo, F.; Higuchi, T. Structural Elucidation of Bamboo Lignin by Acidolysis and Ozonolysis I. *Wood Res.* **1975**, *58*, 1−11.
(43) Bayerbach, R.; Meier, D. Characterization of the water-insoluble fraction from fast pyrolysis liquids (pyrolytic lignin). Part IV: Structure elucidation of oligomeric molecules. *J. Anal. Appl. Pyrolysis* **2009**, *85* (1−2), 98−107.
(44) Balousek, P.; McDonough, T.; McKelvey, R.; Johnson, D. The effects of ozone upon a lignin model containing the beta-aryl ether linkage. *Sven. Papperstid.* **1981**, *84*, R49−54.
(45) Kuitunen, S.; Kalliola, A.; Tarvo, V.; Tamminen, T.; Rovio, S.; Liitiä, T.; Ohra-aho, T.; Lehtimaa, T.; Vuorinen, T.; Alopaeus, V. Lignin oxidation mechanisms under oxygen delignification conditions. Part 3. Reaction pathways and modeling. *Holzforschung* **2011**, *65* (4), 587−599.
(46) Qi, S.; Hayashi, J.; Kudo, S.; Zhang, L. Catalytic hydrogenolysis of kraft lignin to monomers at high yield in alkaline water. *Green Chem.* **2017**, *19* (11), 2636−2645.
(47) Ragnar, M.; Eriksson, T.; Reitberger, T.; Brandt, P. A new mechanism in the ozone reaction with lignin like structures. *Holzforschung* **1999**, *53* (4), 423−428.

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765

(48) Eriksson, T.; Gierer, J. Studies on the Ozonation of Structural Elements in Residual Kraft Lignins. *J. Wood Chem. Technol.* **1985**, *5* (1), 53−84.
(49) Deslongchamps, P.; Moreau, C. Ozonolysis of acetals. (1) Ester synthesis, (2) THP ether cleavage, (3) selective oxidation of *β*-glycoside, (4) oxidative removal of benzylidene and ethylidene protecting groups. *Can. J. Chem.* **1971**, *49* (14), 2465−2467.

DOI: 10.1021/acssuschemeng.8b04856
ACS Sustainable Chem. Eng. 2019, 7, 4755−4765